Development of sampling and bioselective techniques for on-line clinical biosensors

Kaptein, Wilhelmina Anneriek

Chapter 4

Continuous ultraslow microdialysis and ultrafiltration for subcutaneous sampling as demonstrated by glucose and lactate measurements in rats[1]

Voor een succesvolle technologie moet de realiteit voorrang krijgen boven public relations, want de natuur kan niet voor de gek gehouden worden (Richard Feyman, Nobelprijswinnaar 1965).

[1] Kaptein, W.A., Zwaagstra, J.J., Venema, K. and Korf, J. Submitted

Classical microdialysis has some drawbacks. Two main issues in this respect are the time consuming calculations (due to partial recovery of an analyte) and depletion near the sampling site. In this paper we describe a new sampling method, called ultraslow microdialysis (usMD), and compare this with ultrafiltration (UF) at flow rates between 100-300 nl min^{-1}. For the usMD, the recovery at the applied flow rates was 100%. For UF, the flow rates *in vitro* and *in vivo* were the same. As an example of an application of this method, we conducted in a dual enzyme analysis for simultaneous measuring of the glucose and lactate concentration using these sampling techniques in the subcutaneous (sc) tissue of the rat. Both sampling techniques have the potential of on-line *in vivo* monitoring, as well as the measurement of time profiles of analytes by continuous collection and off-line analysis afterwards.

Introduction

Biochemical constituents in blood can serve as parameters for illness, drug metabolism and regulation of homeostasis. Blood sampling and subsequent laboratory analysis is labour intensive, patient unfriendly and often expensive. *In vivo* applied biosensors may overcome many of these problems (Gilbert and Vender, 1996; Anderson et al., 1997). However, until now, there are only few examples of such devices used (batch-wise) in clinical practice. A problem of the biosensors placed in direct contact with the body is that they often evoke a physical reaction, such as covering of the sensor with collagen (Reach and Wilson, 1992) or, when placed in the blood stream, with fibrin (Ash et al., 1992). This creates an additional diffusion barrier, which results in lower measured analyte concentrations (Fischer et al., 1994). In addition, calibration of the sensor in-situ in man is often difficult. Furthermore, a sensor placed *in vivo* often becomes unstable. Measurements with the sensor *in vivo* are, therefore, often inaccurate (Pickup, 1993; Johne et al., 1995).

With microdialysis (MD), relatively clean samples can be obtained. MD is a dynamic sampling method based on analyte diffusion across a semi-permeable membrane driven by a concentration gradient. (Palmisano et al., 1997). MD probes have successfully been applied in both laboratory animals and in man, for example in the brain (Kanthan et al., 1995; Paez et al., 1996), subcutaneous tissue (Lonnroth et al., 1987; Arner et al., 1988) and blood (Telting-Diaz et al., 1992; Stjernstrom et al., 1993; Chen and Steger, 1993; Paez and Hernandez, 1997). A drawback of the MD technique is that calculation of the recovery *in vivo* is difficult, labour intensive and often rather imprecise (Justice, Jr. 1993; Johne et al., 1995). In addition, the sampling can lead to a local depletion of the analytes, because of a removal of the analytes near the MD probe, which leads to an underestimation of the concentrations (Petyovka et al., 1995). To reduce the drainage and to obtain a (near) 100% recovery, the flow of the dialysis fluid has to be very low (less than 0.5 µl min^{-1} (Petyovka et al., 1995)).

Recently (Moscone et al., 1996a; Kaptein et al., 1997a), we have introduced an on-line ultrafiltration (UF) method as an alternative for continuous sampling. UF is a sampling technique that withdraws fluid from the tissue through a semi-permeable membrane. Others (Linhares and Kissinger, 1992; Linhares and Kissinger, 1993; Scheiderheinze and Hogan, 1996) have previously described UF for off-line analysis. The driving force in this process is underpressure in the sampling tube, which creates an influx of extracellular fluid through

the semi-permeable membrane of the probe. We have applied UF for subcutaneous and intravenous sampling in rats with an ultra-slow continuous flow (100 nl min^{-1}) in combination with direct analysis of the glucose content (Moscone et al., 1996). Also, we analysed a concentration profile in a continuously collected 24 hours sample (Kaptein et al., 1997a). The underpressure was created with a disposable syringe, a monovette, with a fluid restriction to ensure a constant flow over several hours.

When we tested the UF in sc tissue of healthy human volunteers (Tiessen et al., 1997), we occasionally observed an additional resistance over the probe, leading to a decrease in the flow rate. Even at flow rates as low as 100 nl min^{-1}, there was insufficient formation of the extracellular fluid. Therefore, in this study we investigated ultraslow microdialysis (usMD) sampling in the subcutaneous tissue of the rat and compare the sampling with ultrafiltration (UF). At flow rates between 100-300 nl min^{-1}, we study the recovery of the usMD, and for UF we evaluate resistance over the probe. We compared the sampling techniques using a dual enzymatic analysis technique for glucose and lactate.

Material and methods

General description of the system

The experimental set-up of a measurement is shown schematically in Fig. 4.1. The probe (for sampling) was coupled to a dual detection system, consisting of an HPLC pump (HPu), a splitter (S; 50-50% splitting ratio) and two enzyme reactors (L and G), both upstream of an electrochemical detector (ECD1 and ECD2). One of the enzyme reactors contained horseradish peroxidase, HRP and glucose oxidase, GOD, whereas the other contained HRP and lactate oxidase, LOD.

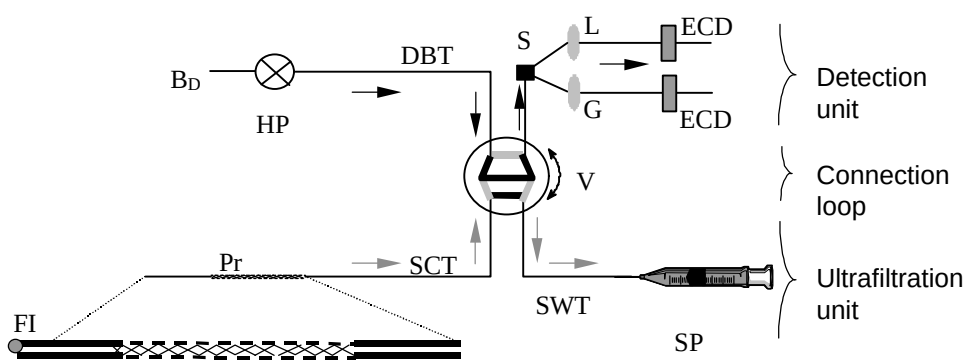

Fig. 4.1. Schematic set-up of the dual enzyme UF/usMD system. BD: buffer detection unit; HPu: HPLC pump detection unit; DBT: detection buffer tube; S: splitter; L: LOD/HRP enzyme reactor; G: GOD/HRP enzyme reactor; ECD1,2: electrochemical detector; V: valve; SCT: sample connection tube; SWT: sample waste tube; BMD: buffer for usMD; FI: fluid inlet for usMD/UF transition; Pr: probe; SP: syringe pump sample unit; =>: flow direction.

Instrumental set-up

Our probe was a modified design of a previously described ultrafiltration probe (Moscone et al., 1996; Kaptein et al., 1997). We used a four centimetre long probe from semi-permeable membrane (fibers of an artificial kidney, AN69HF Filtral 16; Hospal Ind., Meyzieu, France, outer diameter = 290 µm, inner diameter = 240 µm) with a hand-made spring inside (stainless steel wire; Vogelsang, Hagen, Germany; 60 µm diameter, axial length 12 windings cm^{-1}) to prevent collapsing of the fibre. This probe was connected on both ends to a 20-30 cm long fused silica tube (inner diameter 50 µm, outer diameter = 150 µm, Applied Science Group, Emmen, The Netherlands). The spring was glued to the fused silica tubes, and the connection of this with the fibre was closed with cyano-acrylic glue (Henkel, Nieuwegein, The Netherlands). One end was screwed into the valve of the Trident (Spark Holland, The Netherlands), the other end was closed (UF) or held in a PBS-buffer (usMD). The UF or usMD flow (100-300 nl min^{-1}) was driven by the underpressure of a Hamilton syringe (0.5 ml) by pulling with the Harvard apparatus 22 syringe pump. The valve, switched pneumatically by the Trident (load/inject 15/90 seconds), had a loop of 20 µl, which was only partially filled with the sample.

Glucose and lactate were detected electrochemically using bi-enzyme reactors as described by Elekes et al. for glucose (Elekes et al., 1995). The flow injection system has been described before by our group (Kaptein et al., 1997a). In brief, the buffer used for the detection system is a ferrocene-PBS buffer, containing 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 2.5 mM KH_2PO_4. All the chemicals were of pro-analysis quality and were purchased from Merck, Darmstadt, Germany. To this, 0.5 mM Ferrocenemonocarboxylic acid, (Sigma Chemical Co, St. Louis, MO, USA) and 0.1 volume% Kathon CG (Rhom and Haas, Croydon, UK) was added in double quarts distilled water. An HPLC pump (LKB 2150, Pharmacia Bromma, Sweden) pumped the ferrocene-PBS buffer (bubbled with helium to remove air), with a flow of 0.25 ml min^{-1}. This flow was divided in two separate streams after the injection valve and each was pumped through the one enzyme reactor and electrochemical cell. In the enzyme reactor for glucose, 250 U GOD (EC 1.1.3.4, grade I) and 250 U HRP (EC 1.11.1.7), obtained from Boehringer Mannheim (Germany), was immobilized between 0.01 µm cellulose nitrate filters (thickness 100 µm; pore size 0.01 µm, cut-off 50 kDa, Sartorius, Göttingen, Germany). The enzyme reactor for lactate comprised of 25 U LOD and 250 U HRP. The electrochemical cells were thin layer-type cells, with a glassy carbon working electrode hold at 0.00 mV relative to an Ag/AgCl reference electrode and a Teflon/carbon counter electrode (Amor, Spark Holland, Emmen, The Netherlands).

Theoretical considerations for the interpretation of the measurements

To explain the different interventions in the system and the effects from these on the signal, we show a schematic representation of a recorder output for one analyte with these changes (Fig. 4.2).

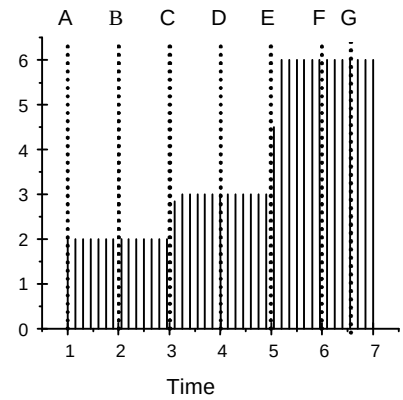

Fig. 4.2. Schematic representation of the different interventions of an in vivo *experiment. A. probe* in vitro*; B. probe* in vivo*; C. sc sample in detector; D. UF => usMD; E. usMD sample in detector; F. flow increase 2-fold; G. high flow sample in detector See text for details.*

At t=1, the measurement starts with analysing a buffer containing a certain concentration of the analyte (glucose or lactate) with the detection system. In this hypothetical case, we assume a start with UF. At t=2, the probe (Pr in Fig. 4.1) is transferred from *in vitro* to subcutaneous tissue. The sample connection tube (SCT) is then still filled with sample from the (*in vitro*) buffer. In case of any change in flow (e.g. as a result of a restriction over the probe membrane), the signal will immediately change. At t=3 the sample of the subcutaneous tissue reaches the detector. In this case, the concentration of the analyte is higher than in the previous buffer. At t=4, the fluid inlet (FI) for usMD is opened, thus starting dialysis. Again, if this would influence the flow rate because of restriction on the probe during UF, this would result in a change in signal. At t=5, the usMD sample reaches the valve (V), thus injected and analysed. If the recovery of the microdialysis is lower than 100%, the signal will decrease. At t=6, the flow is increased two-fold. This leads to a two-fold increase of signal, if the signal increase has a linear relationship with the sample amount. At t=6.5, the first sample from the higher flow reaches V. Changes in recovery at the higher flow, will be shown at this time.

In vitro *experiments*

The calibration of the measurement was performed by placing a probe in the ferrocene buffer with glucose concentrations of 0, 2.5, 7.5 and 10 mM, or lactate 0, 1, 2 and 4 mM, was changed incrementally. The load/inject time was 15/90, therefore the sample was measured every 105 seconds with the ECD and the currents (in nA) were recorded. The calibration curve was made with UF and usMD, which determined the system delay (the lag-time) that was caused by the volume of the connecting tube from the probe to the analysis system and the sensitivity for the analytes.

In vivo *experiments*

Male Wistar rats (250-350 g, Harlan, Zeist, The Netherlands) were housed groupwise on a 12-12h light/dark regime. Their food and drink were provided ad libitum. The rats were anaesthetized by an intraperitonial (i.p.) injection of 6 mg kg^{-1} body wt pentobarbital sodium (Sanofi, Maassluis, The Netherlands) and remained anaesthetized with these drugs. Using a rectal temperature probe and a heat pad, the body temperature was maintained between 36.5 and 37.5 °C.

Before starting an *in vivo* experiment, the sensitivity of the system was determined *in vitro*, using a buffer with 2.5 mM glucose and 1 mM lactate. The probe was placed in sc tissue on the back of the rat with a 16G catheter needle. The flow of the sampling system varied from 100-300 nl min^{-1} by changing the flow rate of the syringe pump (SP). The probe was alternated between UF and usMD by blocking or unblocking the fluid inlet (FI) with a stopper.

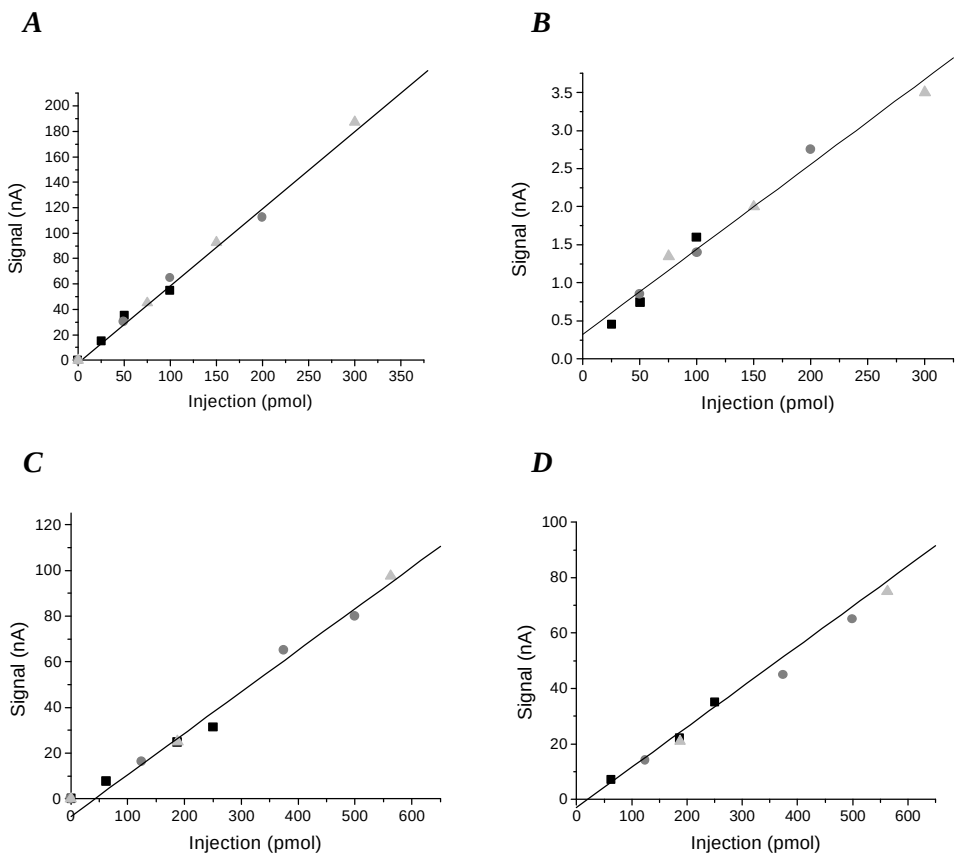

Fig. 4.3. Calibration curves A. lactate, UF (r>0.99); B. lactate, usMD (r>0.98); C. glucose, UF (r>0.99); D. glucose, usMD (r>0.99) ■ *:100 nl* min^{-1}; ● *: 200 nl* min^{-1};▲*: 300 nl* min^{-1}; ——*: regression.*

Results

In vitro *experiments*

Fig. 4.3 shows the effect of a different flow rate on the electrochemical signal for lactate (A&B) and glucose (C&D). For UF (Figs A&C) as well as usMD (Figs B&D) the current was linearly correlated to the concentration of the analyte. The sensitivity for lactate was 0.6 A mmol $^{-1}$ for UF ($r>0.99$; $p<0.000$) and 0.01 A mmol $^{-1}$ for usMD ($r>0.98$; $p<0.000$). The difference in sensitivity for lactate with UF or usMD was caused by the different enzyme activity. The enzyme activity for LOD, and therefore its electrochemical signal, decreases considerably with time. However, the decrease during an (*in vivo*) experiment was negligible, as checked with standards before and after the experiment. For glucose, the sensitivities were respectively 0.18 A mmol $^{-1}$ ($r>0.99$; $p<0.000$) and 0.12 A mmol^{-1} ($r>0.99$; $p<0.000$). The curve remained linear for all curves for the applied amounts from 0-350 pmol for lactate and 0-750 pmol for glucose.

In vivo *experiments*

Fig. 4.4 shows representative data of a measurement in the sc tissue of a rat. As was indicated, the flow was continuously changing and the sampling method (usMD or UF) was alternating. The transition from UF to usMD and vice versa did not change a signal either directly or after the delay caused by the volume of the SCT. This implies that the UF sampling does not have a significant restriction over the probe and that the recovery for this *in vivo* usMD at the applied flow rates is 100%. There was some slight fluctuation in the flow, which can be seen when both analytes fluctuate simultaneously, e.g. at time interval II. This is often seen as a result of irregularities of the pumping system under these extremely low flow conditions. Sometimes, a decrease in flow at UF occurred at the highest flow (300 nl min^{-1}) as a result of the resistance over the membrane of the probe (data not shown).

When the signals for different flows (*in vitro*) were calculated, it appeared that a certain amount of fluid leaked, probably in the valve. This can be seen in Fig. 4.5, when the average of three measurements of glucose before and after a change of flow rate is calculated. The leakage is equivalent to 60 nl min^{-1}, thus with the 15 seconds load this would mean 15 nl per injection. The standard curves shown in Fig. 4.3 did not show this, because at that time the valves did not leak. Correction for this leakage did not influence the results. The concentration of glucose and lactate in the rat during the experiment is shown in Fig. 4.5. The glucose concentration is quite constant, whereas the lactate concentration fluctuates with a trend to lower values.

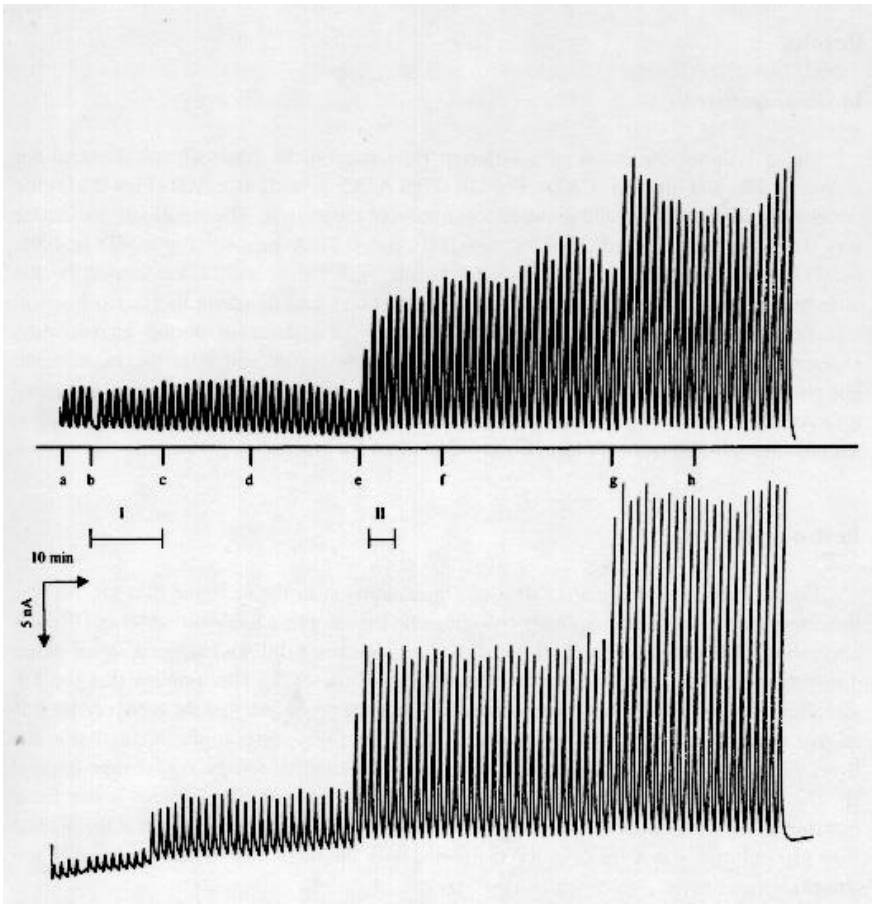

Fig. 4.4. Example of dual enzyme UF/usMD measurement in vivo *for lactate (upper) and glucose (lower). Indicated are: a: probe in UF sampling placed in buffer solution with 2.5 mM glucose and 1 mM lactate; b: probe placed in sc tissue of rat; c: UF sample in detection unit; d: transition UF => usMD; e: flow increase from 100 nl* min^{-1} *=> 200 nl* min^{-1}*; f: transition usMD => UF; g: flow increase from 200 nl* min^{-1} *=> 300 nl* min^{-1}*; h: transition UF => usMD; I: lag-time; II: see text.*

Discussion

In the present study, we demonstrated a system for UF and usMD with different flow rates. To illustrate its application, we coupled the sampling system to a dual enzyme detection system, analysing glucose and lactate simultaneously in one continuous sample in a flow injection analysis system with a splitter. The splitter volume ratio can be changed, and the amount of enzyme in the reactors and the sample volumes injected can also be adjusted. This allows for simultaneous analysis of other constituents, even if different

substrates are added or if sample sizes and sensitivities are different. Leakage in the valve connection must be reduced for these low flows. In combination with a collection tube, as described earlier, profiles can collected and analysed afterwards in laboratory conditions for any analyte, as long as there is an assay with enough sensitivity.

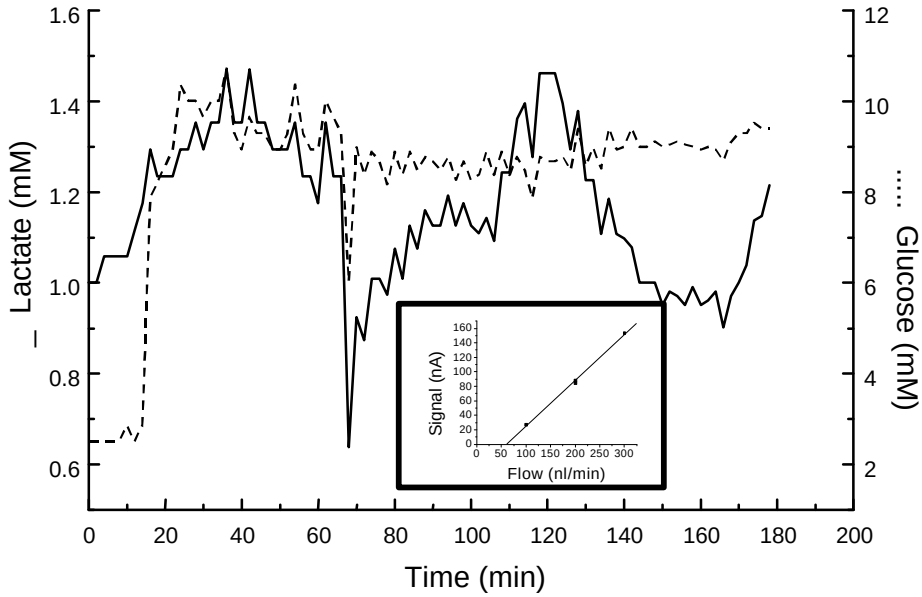

Fig. 4.5. Calculated lactate and glucose concentrations for recorder output of Fig. 4.4. Window: response at different flow rates for glucose in vivo.

The main study object of this research was the comparison of our new continuous sampling system (the ultraslow microdialysis, usMD) to ultrafiltration. By repetitive switches from usMD and ultrafiltration and vice versa, we were able to demonstrate a 100% recovery for this usMD under the applied conditions. We also showed that the UF sampling in the interstitial fluid in the back of the rats can be realized with a flow of 200 nl min^{-1}, and in most cases, such as in the given example, this flow was realized up to 300 nl min^{-1}. As mentioned in the introduction, these findings may be different when the UF technique is used in abdominal tissue in human subjects. When the flow is not measured, correction is difficult.

In the present study, we used a syringe pump, allowing flow changes during the experiment. Unfortunately, at these low flow rates this pump showed some flow disturbances as a result of pulsation caused by the moving parts in the pump. Previously (Moscone et al., 1996; Kaptein et al., 1997), we described a more precise, inexpensive pumping system for fixed ultraslow flow rates: a monovette who's driving force comes from an underpressure and a flow regulation by a fused silica restriction. For the present

experiment this was not utilized because changes of the flow rate are difficult to achieve with this pump.

The low flows for both UF as well as usMD have several advantages. The intervention of the tissue (fluid) around the probe is reduced, because of the small amount of the fluid or analytes removed. In addition, we demonstrated that for usMD the recovery of glucose and lactate is 100% at the applied probe size and flow rates. Labour-intensive, inaccurate recovery calculations are therefore not necessary.

Whether usMD or UF is the best option depends on the *in vivo* application. Both techniques can use disposable material, and both can create the relatively clean samples with (for small analytes) a 100 % recovery. UsMD is the best choice when the amount of fluid at the sampling site is limited. We noticed that in the brain of a rat and in subcutaneous tissue of humans, an *in vitro* flow rate of 100 nl min^{-1} is lower *in vivo* because of a restriction over the membrane of the probe. The exact concentration is then difficult to estimate. The drawback of the usMD technique is the necessity of an additional fluid, making it more difficult to keep the sampling system sterile (especially because the flow is created by the underpressure of the monovette "pump"). When, for example, a 24 hour profile of an analyte of an ambulant patient has to be made, UF will give less risks on infection, so if a constant flow can be created, this is the method of choice. However, when the sampling site is very limited in fluid production, usMD is preferable.

The described usMD and UF techniques can be integrated in many selection and detection systems. Selection can be performed in flow injection systems. The undiluted samples can then be separated with microtechniques, such as capillary electrophoresis (Hadwiger et al., 1997; Dawson et al., 1997) and microbore HPLC (Steele and Lunte, 1995). The absence of large molecules enables direct analysis. Selection can also be performed with enzymes creating products from the analyte which are detectable. An example for this is the enzymatic conversion of glucose into (among other) hydrogen peroxide, an electrochemical detectable compound (Fang et al., 1997; Anderson et al., 1997). This enzymatic reaction is often used in biosensors. Other biosensor selection devices are based on immunological reactions (Morgan et al., 1996b; Kaptein et al., 1997b). Biosensors might better function in UF/usMD sampling devices than in direct contact with the body, because of the relatively "clean" sample, whereas calibration (because of the 100% recovery) is easier than in MD sampling with µl min^{-1} flow rates. The total integration of the sampling, selection and detection in on-line biosensors, as demonstrated by Rigby et al. (1996), using a cheap, pulse-free pumping system as we described earlier (Moscone et al., 1996a; Kaptein et al., 1997a) enables on-line bedside measurements.

In summary, we described a sampling probe for ultraslow microdialysis or ultrafiltration. With simultaneous glucose and lactate analysis, we demonstrated a 100% recovery for microdialysis *in vivo* in subcutaneous tissue of the rat at a flow rate of 100-300 nl min^{-1}. No restriction over the probe was encountered at UF sampling up to at least 200 nl min^{-1}. Both sampling techniques enable on-line *in vivo* measurements as well as sample collection to study time profiles of various compounds by subsequent analysis of the collected sample and can be combined with several microanalysis systems.

References

Anderson, D.J., Guo, B., Xu, Y., Ng, L.M., Kricka, L.J., Skogerboe, K.J., Hage, D.S., Schoeff, L., Wang, J., Sokoll, L.J., Chan, D.W., Ward, K.M. and Davis, K.A. (1997) Clinical chemistry. *Anal. Chem.* **69**, 165R-229R.

Arner, P., Bolinder, J., Eliasson, A., Lundin, A. and Ungerstedt, U. (1988) Microdialysis of adipose tissue and blood for *in vivo* lipolysis studies. *Am. J. Physiol.* **255**, E737-42.

Ash, S.R., Poulos, J.T., Rainier, J.B., Zopp, W.E., Janle, E. and Kissinger, P.T. (1992) Subcutaneous capillary filtrate collector for measurement of blood glucose. *ASAIO. J.* **38**, M416-20.

Chen, Z. and Steger, R.W. (1993) Plasma microdialysis. A technique for continuous plasma sampling in freely moving rats. *J. Pharmacol. Toxicol. Methods* **29**, 111-118.

Dawson, L.A., Stow, J.M. and Palmer, A.M. (1997) Improved method for the measurement of glutamate and aspartate using capillary electrophoresis with laser induced fluorescence detection and its application to brain microdialysis. *J. Chromatogr. B. Biomed. Appl.* **694**, 455-460.

Elekes, O., Moscone, D., Venema, K. and Korf, J. (1995) Bi-enzyme reactor for electrochemical detection of low concentrations of uric acid and glucose. *Clin. Chim. Acta* **239**, 153-165.

Fang, Q., Shi, X.T., Sun, Y.Q. and Fang, Z.L. (1997) A flow injection microdialysis sampling chemiluminescence system for *in vivo* on-line monitoring of glucose in intravenous and subcutaneous tissue fluid microdialysates. *Anal. Chem.* **69**, 3570-3577.

Fischer, U., Rebrin, K., Woedtke, T.V. and Abel, P. (1994) Clinical Usefulness of the Glucose Concentration in the Subcutaneous Tissue - Properties and Pitfalls of Electrochemical Biosensors. *Horm. Metab. Res. Suppl.* **26**, 515

Gilbert, H.C. and Vender, J.S. (1996) The current status of point-of-care monitoring. *Int. Anesthesiol. Clin.* **34**, 243-261.

Hadwiger, M.E., Park, S., Torchia, S.R. and Lunte, C.E. (1997) Simultaneous determination of the elimination profiles of the individual enantiomers of racemic isoproterenol using capillary electrophoresis and microdialysis sampling. *J. Pharm. Biomed. Anal.* **15**, 621-629.

Johne, B., Hansen, K., Mork, E. and Holtlund, J. (1995) Colloidal gold conjugated monoclonal antibodies, studied in the BIAcore biosensor and in the Nycocard immunoassay format. *J. Immunol. Meth.* **183**, 167-174.

Justice, J.B., Jr. (1993) Quantitative microdialysis of neurotransmitters. *J. Neurosci. Methods* **48**, 263-276.

Kanthan, R., Shuaib, A., Griebel, R. and Miyashita, H. (1995) Intracerebral Human Microdialysis: *In vivo* Study of an Acute Focal Ischemic Model of the Human Brain. *Stroke* 870-873.

Kaptein, W.A., Kemper, R.H.A., Ruiters, M.H.J., Venema, K., Tiessen, R.G. and Korf, J. (1997a) Methodological aspects of glucose monitoring with a slow continuous subcutaneous and intravenous ultrafiltrationsystem in rats. *Biosens. Bioelectron.* **12**, 967-976.

Kaptein, W.A., Zwaagstra, J.J., Venema, K., Ruiters, M.H.J. and Korf, J. (1997b) Analysis of cortisol with a flow displacement immunoassay. *Sensors & Actuators B* **45**, 63-69.

Linhares, M.C. and Kissinger, P.T. (1992) Capillary ultrafiltration: *in vivo* sampling probes for small molecules. *Anal. Chem.* **64**, 2831-2835.

Linhares, M.C. and Kissinger, P.T. (1993a) Pharmacokinetic studies using micellar electrokinetic capillary chromatography with *in vivo* capillary ultrafiltration probes. *J. Chromatogr.* **615**, 327-333.

Linhares, M.C. and Kissinger, P.T. (1993b) Pharmacokinetic monitoring in subcutaneous tissue using *in vivo* capillary ultrafiltration probes. *Pharm. Res.* **10**, 598-602.

Lonnroth, P., Jansson, P.A. and Smith, U. (1987) A microdialysis method allowing characterization of intercellular water space in humans. *Am. J. Physiol.* **253**, E228-31.

Morgan, C.L., Newman, D.J. and Price, C.P. (1996) Immunosensors: technology and opportunities in laboratory medicine. *Clin. Chem.* **42**, 193-209.

Moscone, D., Venema, K. and Korf, J. (1996) Ultrafiltrate sampling device for continuous monitoring. *Med. Biol. Eng. Comput.* **34**, 290-294.

Paez, X., Rada, P., Tucci, S., Rodriguez, N. and Hernandez, L. (1996) Capillary electrophoresis-laser-induced fluorescence detection of amphetamine in the brain. *J. Chromatogr. A.* 263-269.

Paez, X. and Hernandez, L. (1997) Blood microdialysis in humans: a new method for monitoring plasma compounds. *Life Sci.* **61**, 847-856.

Palmisano, F., De Santis, A., Tantillo, G., Volpicella, T. and Zambonin, P.G. (1997) Microbial detection by a glucose biosensor coupled to a microdialysis fibre. *Analyst* **122**, 1125-1128.

Petyovka, N., Lyach, L. and Voitenok, N.N. (1995) Homologous ELISA for detection of oligomeric human TNF: properties of the assay. *J. Immunol. Meth.* **186**, 161-170.

Pickup, J. (1993) Developing glucose sensors for *in vivo* use. *Trends. Biotechnol.* **11**, 285-291.

Rigby, G.P., Crump, P.W. and Vadgama, P. (1996) Stabilized needle electrode system for in vivo glucose monitoring based on open flow microperfusion. *Analyst* **121**, 871-875.

Reach, G. and Wilson, G.S. (1992) Can continuous glucose monitoring be used for the treatment of diabetes. *Anal. Chem.* **64**, 381A-386A.

Scheiderheinze, J.M. and Hogan, B.L. (1996) Selective *in vivo* and *in vitro* sampling of proteins using miniature ultrafiltration sampling probes. *Anal. Chem.* **68**, 3758-3762.

Steele, K. and Lunte, C. (1995) Microdialysis sampling coupled to on-line microbore liquid chromatography for pharmacokinetic studies. *J. Pharm. Biomed. Anal.* 149-154.

Stjernstrom, H., Karlsson, T., Ungerstedt, U. and Hillered, L. (1993) Chemical monitoring of intensive care patients using intravenous microdialysis. *Intensive Care Med.* **19**, 423-428.

Telting-Diaz, M., Scott, D.O. and Lunte, C.E. (1992) Intravenous microdialysis sampling in awake, freely-moving rats. *Anal. Chem.* 806-810.

Tiessen, R.G., Kaptein, W.A., Zwaagstra, J.J., Venema, K. and Korf, J. (1998) Glucose kinetics in the human subcutis as assessed with ultrafiltration and frequent analysis. *submitted*